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Drosophila Ring Canal Growth Requires Src and Tec Kinases

Minireview

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One of the many ways cells can communicate with one another is through intercellular bridges that directly link the cytoplasms of sister cells (Robinson and Cooley, 1996). The occurrence of intercellular cytoplasmic bridges is best documented in the germline of both males and females in species ranging from fruit flies to humans. Germline intercellular bridges facilitate the passage of cytoplasmic components between cells during development. Intercellular bridges have also been found connecting somatic cells; however, the function of somatic bridges is not known, nor is the prevalence of somatic bridges established.

During egg development in *Drosophila*, the maternal contribution to each oocyte is supplied largely by 15 nurse cells that are connected to each other and the oocyte by intercellular bridges called ring canals. The nurse cells and oocyte of each egg chamber are sibling germline cells derived from a single precursor through four rounds of mitosis. The mitotic cleavage furrows in these divisions mysteriously halt before daughter cells are completely separated. At the conclusion of mitoses, the arrested cleavage furrows are transformed into ring canals by the addition of an actin cytoskeleton lining the tunnels between cells. Following the generation of egg chambers within the germarium of fly ovaries, egg chambers undergo several days of growth, finally producing eggs almost half a millimeter long. During egg chamber growth, the diameter of ring canals increases from less than 1 μm to about 10 μm (see Figure 1). The large size of both the germline cells and the ring canals, as well as the orderly progression of egg chamber stages within ovaries, permit detailed cytological analysis of ring canals during development.

The identification of ring canal proteins and insight into ring canal biogenesis have been aided by characterization of a class of mutants in which the flow of cytoplasm into the oocyte is impaired (Robinson and Cooley, 1996). The ultimate phenotype of these mutants is striking: egg chambers produce drastically undersized oocytes and the females are consequently sterile. Analysis of these mutants has revealed a pathway for the assembly of the actin cytoskeleton (Figure 2, left). Actin filaments accumulate coincident with HtsRC protein, followed by the addition of Kelch proteins. HtsRC and Kelch functions are not completely characterized; however, it is likely that they are required to maintain or modify the actin cytoskeleton. Before the accumulation of actin, an early indication of ring canal development is the appearance of proteins recognized by antibodies to phosphotyrosine (P-Y) by the end of the fourth mitosis. Later, as actin accumulates, P-Y staining of ring canals becomes quite prominent (Figure 1).

A pathway parallel to the recruitment of actin has emerged from the study of the Src and Tec kinases (Src64 and Tec29; Figure 2, right). Two papers published in *Molecular Cell* (Guarnieri et al., 1998; Roulier et al., 1998) show that mutations in Src64 and Tec29 dramatically reduce both P-Y staining at ring canals and the overall size of ring canals. Neither mutant affects the ring canal localization of actin, HtsRC, or Kelch; rather, the ability of the cytoskeleton to expand during ring canal growth appears to be affected. Src64 is present on the plasma membranes of nurse cells, perhaps including the ring canals. In contrast, antibodies directed to Tec29 protein stained ring canals, and this localization pattern was abolished in *Src64* mutant egg chambers. These data suggest that the key contributor to ring canal P-Y epitopes is Tec29, and that Src64 is required for Tec29 localization to ring canals.

The two stories about Src64 and Tec29 had complementary beginnings. After a long search for mutations in Src64, M. Simon and his colleagues were surprised

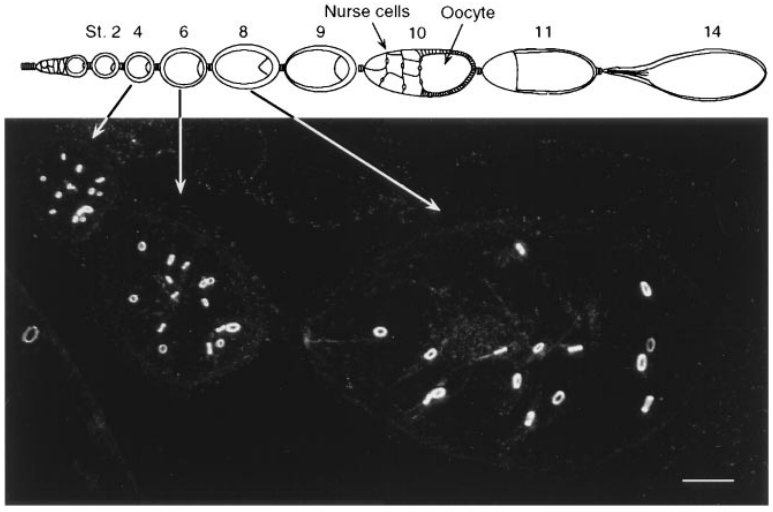

Figure 1. Phosphotyrosine (P-Y) Antibodies Label Ring Canals Intensely

Three egg chamber stages are shown to illustrate the growth of ring canals during oogenesis (scale bar is 10 μm). *Tec29* mutant ring canals have no P-Y staining and ring canal growth is stunted. The upper drawing depicts a single ovariole from an ovary that contains a progression of egg chamber stages (St. 2–14). The stage 10 egg chamber shows the cell types within each egg chamber: nurse cells and oocyte connected by ring canals and surrounded by follicle cells.

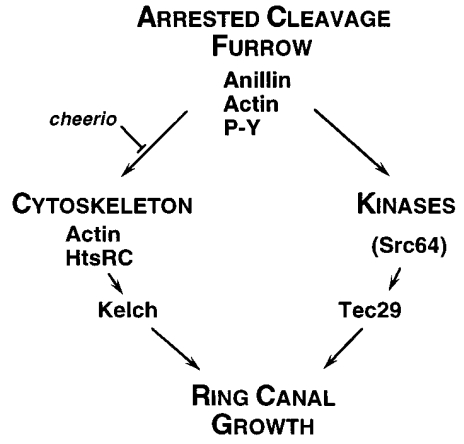

Figure 2. *Drosophila* Ovarian Ring Canal Development

The known cytoskeletal and kinase proteins are recruited independently to arrested cleavage furrows to form ring canals. Both branches are needed to support ring canal growth during oogenesis. *cheerio* mutants block assembly of cytoskeleton. Src64 is shown in parentheses since it is not a prominent ring canal protein, although it is a key regulator of Tec29 localization to ring canals.

to find that nearly complete removal of *Src64* gene expression did not affect the viability of adult animals. The only discernible phenotype was a reduction in female fertility (Dodson et al., 1998). Examination of oogenesis in *Src64* mutant females revealed that many egg chambers failed to complete cytoplasm transfer to the oocyte, and ring canals were abnormally small. In addition, P-Y staining of ring canals was severely reduced. Using the *Src64* mutant as a starting point, Guarnieri et al. (1998) performed a genetic screen to identify mutations in other genes that modified the *Src64* phenotype. One of the enhancing mutations they recovered turned out to be in the *Tec29* gene. Since the *Tec29* mutants were homozygous lethal, oogenesis had to be examined in heterozygous females that harbored homozygous mutant germline cells. Remarkably, oogenesis in these mosaic animals displayed the same phenotype as was seen for *Src64* mutants: incomplete cytoplasm transport and small ring canals.

A central role for Tec29 in ring canals was also discovered by S. Beckendorf and colleagues (Roulier et al., 1998) after their identification of a *Tec29* mutant. This group's initial interest was in the contribution of Tec29 to development, and they found severe defects in head involution during late embryogenesis. This stage of development is quite complicated, making interpretation of the *Tec29* phenotype difficult. To look for phenotypes in younger embryos, mosaic females were constructed with homozygous mutant nurse cells and therefore no maternally supplied Tec29. However, embryos derived from these females had no additional phenotypes beyond what was already observed during head involution in homozygous animals. Instead, an effect on egg size was noticed and this led to the examination of oogenesis and the characterization of the ring canal phenotype.

There is evidence both for the regulation of Tec family kinases by Src family kinases and for the involvement of these classes of protein in regulating actin (see Brown and Cooper, 1996; Thomas and Brugge, 1997). The study of *Drosophila* ring canals offers a new opportunity to explore the roles of these proteins in vivo in a system with facile genetics, excellent cytology, and well characterized cytoskeletal dynamics. Future studies should reveal substrates for Tec29 in egg chambers. Of the known ring canal proteins, Kelch is an obvious candidate for a substrate, even though it has not been determined whether Kelch is phosphorylated. Similarly to *Tec29* mutants, *kelch* mutants affect ring canals after the actin cytoskeleton is established. The biochemical role for Kelch is likely to involve the ability to cross-link actin filaments as they accumulate at the ring canal, possibly in a manner that allows actin filaments to slide past one another during ring canal growth (Tilney et al., 1996; Robinson and Cooley, 1997). Phosphorylation of a subpopulation of Kelch could be required to regulate Kelch interaction with actin or another protein. Tec29 and Kelch localization to ring canals appear to follow a similar pattern in which the proteins are initially present on a subset of canals and appear on all ring canals later in egg chamber development, well after HtsRC and actin are present. Colocalization studies using Tec29 and Kelch antibodies are needed to clarify whether they are recruited to ring canals simultaneously.

Tec29 probably does not account for all of the P-Y staining in egg chambers. P-Y staining begins by the end of the final mitosis, whereas Tec29 localization begins later. Also, germline cells lacking Tec29 retained some P-Y staining. The presence of earlier P-Y epitopes suggests that there is a germline tyrosine kinase substrate that is required to initiate ring canal development. A P-Y protein could be involved with modifying the plasma membrane at the site of arrested cleavage furrows to form a foundation for the ring canal cytoskeleton, reminiscent of the presence of P-Y proteins in junctional complexes in somatic cells (Craig and Johnson, 1996). Src64 may be involved in ring canal initiation since *Src64* mutants have destabilized ring canals (Dodson et al., 1998), or additional tyrosine kinases regulating ring canal development remain to be identified. A candidate for a protein involved with initiation of ring canal development is the product of the *cheerio* gene (Robinson et al., 1997). *cheerio* mutant ring canals do not accumulate actin, HtsRC, or Kelch, although they do have abundant P-Y staining, and the ring canals do not grow properly.

Another interesting question for future work is whether Src or Tec kinases are required for the formation of other intercellular bridges. Sperm development in the male germline of many animal species is syncytial, with clusters of cells connected by ring canals. The *Drosophila* male ring canals stain with P-Y antibodies, suggesting that there could be phosphoproteins similar to those in the female. However, the localization of Tec29 and Src64 to male ring canals was not reported, nor was an effect on male fertility described in *Src64* mutants. In addition to one or more phosphoproteins, male ring canals contain anillin and at least three septins, but no actin (Hime et al., 1996). If Tec29 is a component of male ring canals, it might have a different constellation of substrates than in female ring canals. As intercellular bridges from other

cell types are characterized, it will be interesting to follow the contribution of kinases to their morphogenesis and function.

Selected Reading

Brown, M.T., and Cooper, J.A. (1996). Biochem. Biophys. Acta *1287*, 121–149.

Craig, S.W., and Johnson, R.P. (1996). Curr. Opin. Cell Biol. *8*, 74–85.

Dodson, G.S., Guarnieri, D.J., and Simon, M.A. (1998). Development, in press.

Guarnieri, D.J., Dodson, G.S., and Simon, M.A. (1998). Mol. Cell *1*, 831–840.

Hime, G., Brill, J., Wolf, N., and Fuller, M. (1996). J. Cell Sci. *109*, 2779–2788.

Robinson, D.N., and Cooley, L. (1996). Trends Cell Biol. *6*, 474–479.

Robinson, D.N., and Cooley, L. (1997). J. Cell Biol. *138*, 799–810.

Robinson, D.N., Smith-Leiker, T.A., Sokol, N.S., Hudson, A.M., and Cooley, L. (1997). Genetics *145*, 1063–1072.

Roulier, E.M., Panzer, S., and Beckendorf, S.K. (1998). Mol. Cell *1*, 819–829.

Thomas, S.M., and Brugge, J.S. (1997). Annu. Rev. Cell Dev. Biol. *13*, 513–609.

Tilney, L.G., Tilney, M.S., and Guild, G.M. (1996). J. Cell Biol. *133*, 61–74.